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TRANSLATIONS

AFFIDAVIT OF ACCURACY

I, Jessica Doss, hereby certify that the following is, to the best of my knowledge and belief, a true and accurate translation of the accompanying document in connection with Application DE 103 51 414.7 from German into English.

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Laser Scanning Microscope with a Non-Descanned Detection and/or Observation Beam Path

In Laser Scanning Microscopy, the Non-Descanned Detection method (NDD) is employed frequently. This method is especially important, if highly scattering probes are to be examined or large penetration ranges are to be achieved, and only very closely adjacent mirrors with limited light conductivity are available in the LSM scan head for the detection. In that case, the detection of the excitation to be modulated takes place externally in non-descanned manner in the vicinity of an aperture diaphragm.

Usually, the excitation in the UV/VIS range is generated through a two-photon process with pulsed IR radiation [US 5034613]. The total emitted fluorescence radiation is then assigned to the excitation in the confocal volume of the focus, and can be detected, possibly after scattering several times in the probe, in the reflected light channel or the transmitted light channel. For that, high light conductivity is necessary in the detection channel.

Therefore, a basic requirement in optimized layouts lies in the effective and probe-near detection of the fluorescence radiation, in order to maximize the effective field of vision as the virtual source of scattered light. The accessibility in these regions is however frequently limited due to the interfaces of the microscope stand itself, as well as due to the supplementary devices used, like the pipettes and the electrodes. A significant boundary condition is given by the detector itself, which exhibits not only spectral but also positional and angle-dependent sensitivities.

Further, one must assume that the remaining microscope optics to be used is set up for a regular beam path, and has a bounded free diameter. In order to prevent trimming of the scattered light at the borders of the subsequent optical system, such as the tube lens or the illumination optics in the reflected light channel, the excitation and the emission must be separated in the reflected light beam path near the objective. In general,

"push & click" beams splitters are used by default in the reflector plane of the mirror in the reflected light channel in order to maintain maximum possible flexibility. The boundaries of the subsequent regular layout of the optical system are largely taken for granted hitherto. In particular, it is of advantage if the defined interfaces, such as the TV port, are used for the coupling in the detection module. With the position and the size of such an interface, however, most of the main boundary conditions for the transmission of the stray light are fixed.

The aim of the claimed arrangement lies in modifying the beam path immediately after the separation of the excitation and the detection of the beam path in such a manner that the maximum possible (untrimmed) visual field can be imaged onto the detector, taking into consideration the subsequent optical arrangement in the beam path.

This problem is solved by the features described in the claim 1. Other preferable enhancements in the embodiment are the subject matter of the dependent claims.

One such advantageous embodiment involves positioning of an additional optical arrangement, preferably comprising a convex lens in the small reflector frame of the reflected light channel mirror. Its dimensions are determined on the basis of a compromise between the maximum deflection of the main beam and the resulting not-too-large diameter of the bundle. In particular, the optimization can take place in such manner that the NDD module can be used at a TV port.

Figure 1 explains the effect of the arrangement for a beam path. Shown is a part of the beam path in a Laser Scanning Microscope (see, for instance DE 197 02 753 A1), from a scanner aperture diaphragm SP to a scan objective SO for transmission of the illumination light beam, an illumination tube lens 5 and a beam splitter 1 (main dichroic beam splitter for separating the excitation and the detection beam) in the direction of the objective (only the objective aperture diaphragm 3 is shown here).

A non-descanned detection beam path passes through 1 and a mirror 2 as well as a detection tube lens 4 in the direction of the detection, whereby another beam splitter ST 3 can be provided for masking the illumination beam path. On insertion of a convex lens (6) at the reflector (1) immediately after the reflection, the normally used diameter of the reflector (2) is reduced, thus enabling greater transparency for the scattered light. One can see that, in particular, the normally used diameter before the illumination tube lens (4) is distinctly greater compared to that of detection tube lens (5), which are each at the same distance from the objective, and thus directly illustrates the derived benefit. In the example shown, the aperture at the border area can be increased by about 15%, corresponding to about 30% increase in the brightness.

The convex lens can be inserted with advantage immediately on the reflector at the reflector housing, for instance, at a slot of insertion, whereby the available slots for the insertion of the filters can also be used. It is advantageous if the lens is also replaceable or can be plugged in and out.

It can also be arranged in the direction of the detection before the reflector 2 at its reflector housing.

A second lens can also be provided at the mirror 2 or can be integrated in the mirror housing, singly or in combination with a lens at the beam splitter. It can also comprise a tilted mirror in a deflecting element in the form of a convex or a concave mirror.

Patent Claims

1.
Laser Scanning Microscope with a non-descanned detection and/or observation beam path, wherein a beam splitter is provided for separation of the illumination and detection beam path and in the direction of the detection at least one optical arrangement for regular transmission of the detected light is provided,
whereby between the beam splitter and the optical arrangement, an additional optical arrangement is provided for reducing the diameter of the bundle of the beam to be imaged.

2.
Laser Scanning Microscope according to claim 1,
whereby the additional optical arrangement is a convex lens.

3.
Laser Scanning Microscope according to claim 1,
whereby the additional optical arrangement is embodied as a diffractive optical element (DOE).

4.
Laser Scanning Microscope according to claim 1, 2 or 3,
whereby the additional optical arrangement is mounted immediately at the beam splitter housing in the direction of the detection.

5.
Laser Scanning Microscope according to one of the claims 1-4,
whereby the additional optical arrangement is integrated in the housing of the beam splitter.

6.
Laser Scanning Microscope according to one of the claims 1-5, whereby the additional optical arrangement is replaceable or can be plugged in.

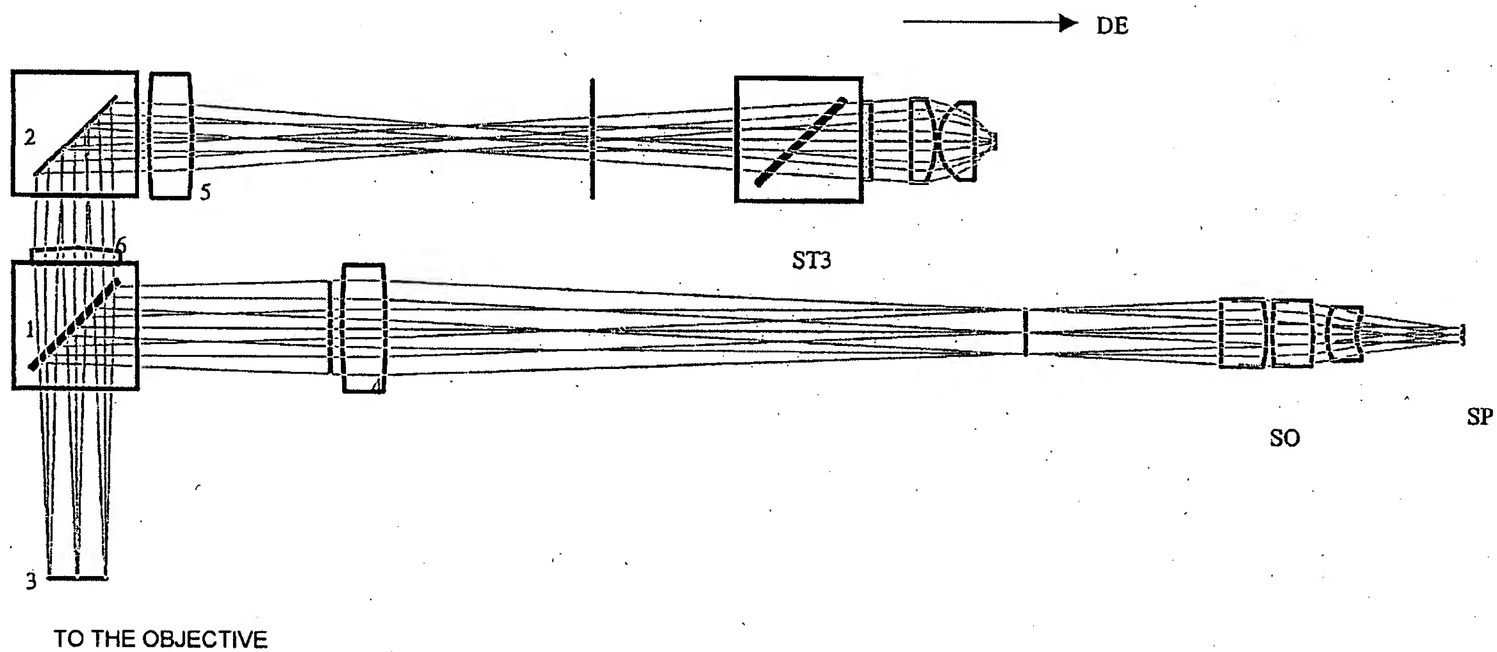
7.

Laser Scanning Microscope according to one of the claims 1-6,
whereby a second lens is provided on an another deflecting element or is integrated in the latter, singly or in combination with the additional element at the beam splitter.

8.

Laser Scanning Microscope according to one of the claims 1-7,
with an embodiment comprising a tilted mirror in an deflecting element in the form of a convex or concave mirror.

FIG. 1



Summary

Laser Scanning Microscope with a non-descanned detection and/or observation beam path, wherein a beam splitter is provided for the separation of the illumination and the detection beam paths and in the direction of the detection, at least one optical arrangement is provided for the regular transmission of the detected light, whereby between the beam splitter and the optical system, an additional optical arrangement is provided for reducing the diameter of the bundle of the beam to be imaged.